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INTELLECTUAL PROPERTY / TECHNOLOGY LAW
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EXAMINER
DAVIS, MINH TAM B

ART UNIT	PAPER NUMBER
1642	17

DATE MAILED: 08/11/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/878,454	MONTEIRO ET AL.	
	Examiner	Art Unit	
	MINH-TAM DAVIS	1642	

-- The MAILING DATE of this c mmunication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 April 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disp sition of Claims

4)☒ Claim(s) *1,2,4-7 and 9-32* is/are pending in the application.

4a) Of the above claim(s) *5-7,10,11,13-22,26 and 29-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4,9,12,23-25,27 and 28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Applicant cancels claims 3, 8 and adds new claims 26-32.

Applicant asserts that the original election is directed to group 29, the composition claim 12.

After review and reconsideration, the composition claim 12 and related new claims 27-28 are examined together with the method claims 1-2, 4, 8-9, 23-24 and related new claim 25.

Further, since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, new claims 26, 29-32 are withdrawn from consideration as being directed to a non-elected invention for the following reasons (See 37 C.F.R. 1.142(b) and M.P.E.P. 821.03):

Claim 26, drawn to an *in vitro* method to determine the effectiveness of a test compound to reduce apoptosis comprising administering the calcium binding protein of SEQ ID NO:2 to cultured cells and contacting the cultured cells with the test compound, differ from the methods of reducing apoptosis comprising administering a mutant of the the calcium binding protein of SEQ ID NO:2 of claims 1-4, 8-9, 23-25 in method objectives, method steps, reagents and/or dosages, and/or schedules used.

Claim 29 is drawn to a distinct mutant with substitutions at both the non-elected N-terminal amino acid position 2 and at the amino acid 172 of the non-elected calcium-binding EF-hand comprising amino acids 161-173 of SEQ ID NO:2 , wherein the

property and characteristic of the said mutant is different from a mutant of the elected claim 12 having a mutation in the amino acid residues 116-128 in the calcium-binding EF-hand of SEQ ID NO:2.

Claims 30-31 are drawn to an expression vector, which has different properties than the protein mutant of claim 12.

Claim 32 is drawn to an antibody that binds to SEQ ID NO:2, which has different structure and function as compared to the protein mutant of claim 12.

Accordingly, claims 1-2, 4, 9, 12, 23-25, 27-28, a mutation in the amino acid residues at position 116-128 in the EF calcium binding hand only are examined in the instant application.

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH, SCOPE

Claims 1-2, 4, 9, 23-24 remain rejected under 35 USC 112, first paragraph pertaining to lack of enablement for an "*in vivo*" method for reducing apoptosis, or an *in vitro* method for inducing apoptosis, comprising contacting cells with a mutant calcium-binding protein to "effect or to inhibit interaction of presenilin of SEQ ID NO:1 with said mutant calcium binding protein", wherein the substitution is at "any amino acid" at positions 116 to 128 of SEQ ID NO:2, using "any amino acid" for the substitution. New claim 25, and the composition claims 12, 27-28 used in said method are rejected for the same reasons already of record in paper No:15.

Applicant argues as follows:

Concerning lack of correlation between familiar Alzheimer's Disease (AD) and induced apoptosis caused by interaction between presenilin 2 (SEQ ID NO:1) and calmyrin (SEQ ID NO:2 or calcium binding protein), Applicant asserts that it is well established that presenilin 2 is implicated in the regulation of programmed cell death, and causes the majority of early onset of familiar Alzheimer's Disease. Applicant asserts that the instant invention shows that protein-protein interaction between presenilin 2 and calmyrin causes an increase in apoptosis as compared to the apoptosis caused by presenilin 2 alone, and that by mutating specific regions of calmyrin, the protein-protein interaction is reduced and apoptosis is reduced.

Applicant argues that Hela cells have been used investigating drugs and other compounds, because there is a clear correlation between results found *in vitro* and *in vivo* testing models. Applicant asserts that for example 1) Navarova et al teach that the testing results of the cardioprotective drug stobadine are comparable between *in vitro* results in Hela cells and *in vivo* results in mice, 2) Macickova et al teach that induced lysosomal enzyme activity is comparable between *in vitro* results in Hela cells and *in vivo* results in rats, 3) Okubo et al teach that *in vitro* cytotoxicity testing results in Hela cells are comparable to *in vivo* results in rabbits, 4) Nobuyuki et al teach that correlation between toxicity induced by chromate found in an *in vivo* testing model and *in vitro* results in Hela cells, and 5) Ookata et al teach that the same phosphorylation sites are found in both *in vitro* testing using Hela cells and *in vivo* tests.

Applicant asserts that the cells in culture recited by the Examiner are primary or secondary cell culture, which is different from and cannot be compared with Hela cells,

which are immortalized cells and not oncogenic in animals unless transformed by a virus. Applicant asserts that the Hodgkin's disease HD cell lines taught by Drexler et al are not immortalized cells. Applicant asserts that the Embleton reference is not relevant because it is related to lack of antigens on cultured cells, thereby reducing the accuracy of interpreting results obtained with monoclonal antibodies, and because the present invention does not include the production of antibodies for Hela cells or tumor cells, but instead antibodies are raised for epitope on calmyrin. Applicant asserts that Freshney et al states that many inconsistency in the cell cultures can be rectified by inclusion of a number of different hormones in the culture media. Applicant asserts that the reference by Hsu et al is not relevant, because Hsu et al discuss different analysis for monitoring cell population, by the chromosome constitution of the in vitro cell line, and because the present invention monitor the level of apoptosis by a simple procedure that can include manual counting of dead cells.

Applicant recites *Ex parte Balzarine*, stating that the Appellant could provide evidence that established that those skilled in the art would accept the *in vitro* testing would be useful in *in vivo* treatment of humans.

Applicant asserts that a precise location of the binding site is not necessary to establish enablement, since it is enough that when calmyrin is mutated in the disclosed region, the binding affinity is reduced and in fact the level of apoptosis is reduced.

Applicant further asserts that the Examiner contends that the decrease in apoptosis is not predictable because the mechanism of cell death level is not known, and the mutation in calmyrin cannot be predicted to be the region necessary for binding

to presenilin 2. Applicant asserts that it is not a requirement for patentability that the inventor knows how or why an invention works.

Concerning the Examiner statement that the scope of the claims includes a method for reducing apoptosis using numerous structural variants, Applicant asserts that the specification discloses the exact regions for mutations in the calmyrin protein that have been found effective to reduce binding affinity with the presenilins. Applicant asserts that these regions include the calcium binding EF hands of SEQ ID NO:2, including amino acid residues at positions 116 to 128 and 161 to 173, and the N-terminal of SEQ ID NO:2. Applicant asserts that mutation of the calcium binding EF hands of calmyrin reduces the binding affinity between the calmyrin and presenilins thereby reducing apoptosis. Applicant asserts that one can easily cause a mutation in either the calcium-binding EF-hands or at the 1-3 amino acid residues at the N-terminal of calmyrin.

The recitation of Navarova et al, Macickova et al, Okubo et al, Nobuyuki et al, Ookata et al and the case law *Ex parte Balzarine* is acknowledged.

Applicant's arguments in paper No:16 have been considered, but are found not to be persuasive for the following reasons:

1) Contrary to Applicant's assertion, Applicant has not shown that protein-protein interaction between presenilin 2 and calmyrin "causes" an increase in apoptosis as compared to the apoptosis caused by presenilin 2 alone, and that "by mutating specific regions of calmyrin, the protein-protein interaction is reduced and apoptosis is reduced".

Although the specification discloses that presenilin 2 and calmyrin seems to be colocalized in the ER when cotransfected in Hela cells, and although cotransfection of these two proteins enhances apoptosis, as compared to apoptosis caused by each protein by itself, this does not necessarily mean that protein-protein interaction between presenilin 2 and calmyrin "causes" an increase in apoptosis as compared to the apoptosis caused by presenilin 2 alone, because the mechanisms of apoptosis caused by either protein alone are not known, and several different, unrelated factors could potentially effecting apoptosis caused by presenilin 2, such as perturbations of calcium, oxidative stress, increased signaling by heterotrimeric GTP-binding proteins and G1 cell cycle arrest have been implicated (specification, page 4, second paragraph), and could indirectly affected by one of the transfected protein, such as the level of calcium as related to the presence of the calcium binding protein calmyrin.

In addition, contrary to Applicant's assertion, the specification does not disclose the regions for mutations in the calmyrin protein that have been found "effective to reduce binding affinity" with the presenilins. There is no indication that reduction in apoptosis in Hela cells by administration of the mutant EF-N of the calmyrin (calcium binding protein) of SEQ ID NO:2 is in any way correlated with inhibition of the interaction between presenilin 2 and said mutant.

It is noted that it is a misinterpretation of the Examiner position when Applicant recites that "the Examiner contends that the decrease in apoptosis is "not predictable" because the mechanism of cell death level is not known, and the mutation in calmyrin cannot be predicted to be the region necessary for binding to presenilin 2".

Rather, the Examiner stated in previous Office action that it is unpredictable that the mutant EF-N of SEQ ID NO:2 would facilitate or effect the "inhibition of the interaction between PS2 of SEQ ID NO:1 and the calcium binding protein or calmyrin of SEQ ID NO:2 (previous Office action, p.12, second paragraph).

The Examiner has noted that the specification discloses that cotransfection of PS2 and the mutant EF-N of calmyrin or SEQ ID NO:2 reduces apoptosis level as compared to the apoptosis level caused by transfection of PS2 alone. Further the Examiner agrees that usually it is not a requirement for patentability that the inventor knows how or why an invention works. However, as written the claims encompass a method to reduce induced apoptosis, wherein said reduction in apoptosis is mediated by inhibition of the interaction of presenilin 2 (PS2) of SEQ ID NO:1 and the mutant EF-N of the calcium binding protein or calmyrin of SEQ ID NO:2 . In other words, the mechanism of reduction of induced apoptosis is claimed as caused by inhibition of the interaction of presenilin 2 (PS2) of SEQ ID NO:1 and the mutant EF-N of the calcium binding protein or calmyrin of SEQ ID NO:2 . As stated in the previous Office action, it is not known how or why the mutant EF-N of SEQ ID NO:2, when cotransfected with SEQ ID NO:2 reduces apoptosis as compared to the apoptosis induced by SEQ ID NO:2 alone. Moreover, the mechanism by which presenilin induces apoptosis are not fully understood, but several different, unrelated factors have been implicated, such as perturbations of calcium, oxidative stress, increased signaling by heterotrimeric GTP-binding proteins and G1 cell cycle arrest (specification, page 4, second paragraph). Further, the mutation of the mutant EF-N of SEQ ID NO:2 is on the amino acid 127 in

the calcium binding domain, and Applicant has not shown that the calcium binding domain is necessary for the binding and interaction with PS2 of SEQ ID NO:1.

Thus it is unpredictable that reduction in apoptosis, in Hela cells transfected with the mutant EF-N of SEQ ID NO:2 and PS2 of SEQ ID NO:1, as compared to apoptosis caused by PS2 of SEQ ID NO:1 alone is caused by inhibition of the interaction of SEQ ID NO:1 and said mutant.

2) Further, contrary to Applicant's arguments, transfected Hela cells *in vitro* with overexpression of PS2 of SEQ ID NO:1 and the calcium binding protein of SEQ ID NO:2 (calmyrin) having mutation at position 127 in the calcium binding EF hand comprising amino acids 116 to 128 of SEQ ID NO:2 are not a model for *in vivo* cells including neuronal cells that have induced apoptosis due to protein-protein interaction between SEQ ID NO:1 and SEQ ID NO:2 for the following reasons:

A) Except for transfected Hela cells *in vitro*, it is even not clear which cells or which neuronal cells *in vivo* that have induced apoptosis due to protein-protein interaction between SEQ ID NO:1 and SEQ ID NO:2.

Although mutations of PS1 and PS2, especially missense mutations in residues that are conserved between the two proteins are linked to early onset of familial AD, the mechanisms by which mutations in PS1 and PS2 cause AD is not known (specification, page 3, first paragraph). Thus since the mechanisms by which mutations in PS1 and PS2 cause AD are not known, and since PS2 alone could cause apoptosis, albeit less than apoptosis caused by PS2 transfected with calmyrin, one cannot extrapolate that apoptosis in neuronal cells in AD is due to or induced by interaction between PS2 and

calmyrin, wherein said interaction is not germane or related to mutations found in PS2 that may be linked to AD.

Thus except for the transfected Hela *in vitro*, it is not even clear which cells *in vivo* or which diseases have induced apoptosis caused by interaction between PS2 and calmyrin.

B) In transfected Hela cells, SEQ ID NO:1 and the mutated SEQ ID NO:2 are overexpressed, which is not the case with *in vivo* conditions, wherein PS1, PS2 (SEQ ID NO:1) and SEQ ID NO:2 are expressed at low protein level as disclosed in the specification (p.3, second paragraph, and p.29, lines 1-2). How a cell responds to an apoptotic signal however depends on the intracellular concentrations of a particular family members of proteins that are related to apoptosis (Oltvai et al, of record). Thus an increased or reduced apoptosis could be due to artificial increased levels of SEQ ID NO:1 and 2 in transfected Hela cells.

C) There is no homeostasis regulation and cell-cell interaction in *in vitro* conditions, that can affect the final response and outcome of a cell *in vivo* (Hsu et al, of record). Further response to apoptotic signals, and characteristics and properties of Hela cells are not the same as neuronal cells *in vivo* because Hela cells are not neuronal cells. Applicant did not seem to address this issue.

D) Further, cell culture artifacts are well known in the art, and cells in culture have different characteristics and properties of comparable cells *in vivo* and this difference would be applied to Hela cells as well (Drexler et al, Embleton et al, Hsu et al, Freshney et al, and Dermer et al, all of record). The teaching in the art would be

relevant and apply to any cell in culture, because due to adaptation to cell culture environment, acquisition or loss of certain properties or certain surface antigen proteins, or chromosomal constitution, i.e. acquisition or loss of expression of genes, would profoundly affect the characteristics and properties of the cells when adapted to culture environment.

Further, although there are some similarity between responses to some drugs or to some toxic compounds, or induced levels of some enzyme in Hela cells *in vitro* and *in vivo* models, as recited by Applicant, this cannot be extrapolated to responses of *in vivo* cells to apoptosis signals because *in vivo* responses to different drugs are different and unpredictable, especially when homeostasis regulation and the *in vivo* stability of the mutant of SEQ ID NO:2 are considered, wherein the half life of the mutant of SEQ ID NO:2 (EF-N mutant) used in the claimed method is reduced as compared to the wild type (specification, p.38, second paragraph). There is no indication that the art would accept that *in vitro* inhibition of apoptosis in Hela cells by any compound as predictive of *in vivo* inhibition of apoptosis in any cell.

3) Further, Applicant has not shown how to use the claimed variants having any type of substitution with any amino acids at any amino acid at positions 116 to 128 of the calcium binding EF-hand of SEQ ID NO:2, which are capable of functioning as that which is being disclosed.

Although the specification contemplates mutation of any amino acid in the region of the calcium binding EF hands of SEQ ID NO:2, comprising amino acid residues at positions 116 to 128 of SEQ ID NO:2, the only actual example of mutation is the

substitution from Aspartic acid 127 to Asparagine 127 of SEQ ID NO:2, which is a conservative substitution. Applicant only shows that a single, conservative substitution at amino acid 127 reduces the apoptosis induced by SP2, which cannot be extrapolated to substitutions at any amino acids in the calcium binding EF hand of calmyrin comprising amino acid residues at positions 116 to 128 of SEQ ID NO:2, because the effect of these substitutions on apoptosis are unpredictable. Why and how said substitution at amino acid 127 reduces the apoptosis induced by SP is not known. Applicant has not shown that mutation of any amino acid, including amino acid 127 in the calcium binding EF hand of calmyrin comprising amino acid residues at positions 116 to 128 of SEQ ID NO:2 "reduces the binding affinity" between the calmyrin and presenilins thereby reducing apoptosis.

Further, concerning substitutions at amino acid 127 with any amino acid other than Asparagine 127, and substitutions of amino acids 116-126, 128-129 with any amino acid, the effect of these substitution on the function of SEQ ID NO:2 is unpredictable, because even a single amino acid substitution or what appear to be an inconsequential chemical modification will often dramatically affect the biological activity and characteristics of a protein, as taught by Burgess et al, Lazar et al, Tao et al and Gillies et al, all of record).

In view of the above, the claimed invention lacks enablement for an "*in vivo*" method for reducing apoptosis, or an *in vitro* method for inducing apoptosis, comprising contacting cells with a mutant calcium-binding protein to "effect or to inhibit interaction of presenilin of SEQ ID NO:1 with said mutant calcium binding protein", wherein the

substitution is at "any amino acid" at positions 116 to 128 of SEQ ID NO:2, using "any amino acid" for the substitution.

REJECTION UNDER 35 USC 102(a and b), NEW REJECTION

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims12, 27 are rejected under 35 U.S.C. 102(a and b) as being anticipated by Seki N et al, 1998, Saito T et al, 1999, or Naik, MU et al, 1999, Genbank Sequence Database (Accession No: Q9Z0F4), National Center for Biotechnology Information, National Library of Medicine, Bethesda, Maryland.

Claims12, 27 are drawn to a purified mutant calcium-binding protein, comprising an amino acid sequence as set forth in SEQ ID NO:2 and having a substitution of "at least" one amino acid residue in "at least" one calcium-binding EF-hand of SEQ ID NO:2 (claim 12), wherein the substitution of at least one amino acid residue in at least one

calcium-binding EF-hand of SEQ ID NO:2 "comprises" amino acid residues at positions 116 to 128.

Seki N et al, 1998, Saito T et al, 1999, and Naik, MU et al, 1999 teach an amino acid sequence which is the same as SEQ ID NO:2, except amino acids at positions 124 and 130, under MPSRCH sequence similarity search (MPSRCH search report, 2002, us-09-878-454a-2.rsp, pages 2-3).

The amino acid sequence taught by Seki N et al, 1998, Saito T et al, 1999, and Naik, MU et al, 1999 seems to be the same as the claimed mutant calcium-binding protein.

The office does not have the facilities and resources to provide the factual evidence needed in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art and to establish patentable diffrences. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone

numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS
August 1, 2003

SUSAN UNGAR, PH.D
PRIMARY EXAMINER